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Mapping the essential structures of human ribosomal protein L7 for nuclear entry, ribosome assembly and function

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Abstract **Human large subunit protein L7 carries multiple nuclear localization signals (NLS) in its structure: there are three monobasic partite NLSs at the NH_2-region of the first 54 amino acid residues and a bipartite in the middle section at position of 156–167. The C-region of the last 50 amino acid residues displays membrane binding nature, and might involve in forming a nuclear microbody for pre-nucleolar ribosome assembly. The middle section covers 144 amino acid residues which are essential for the structure and function of ribosome. This is evident from findings that truncated L7 without the NH_2-region or the C-region, or missing both regions, is capable of reaching nucleolus and incorporating in ribosome, however, only ribosomes bearing truncated L7 without the NH_2-region is capable of engaging in polysome formation. Combining with the phylogenic findings from homologous sequence alignment, the NH_2-region of L7, besides being as a eukaryotic expansion segment, can be excluded from building a functional eukaryotic ribosome.**

Keywords: Nuclear localization signal; Eukaryotic ribosomal protein; Ribosome assembly; Ribosome expansion segment; Nuclear microbody

1. Introduction

An intriguing aspect of ribosome assembly in the eukaryotic cell is that it is another level of control for gene expression. Ribosomes are assembled from ribosomal proteins and ribosomal RNAs in the nucleolus, and are then exported to the cytoplasm to carry out translation. To accomplish such a mission, ribosomal proteins, after being synthesized, are imported to the nucleus thence to the nucleolus. The nuclear entry of ribosomal proteins, like other nuclear proteins, depends upon a nuclear localization signal (NLS) and uses cargo/carrier machinery to cross the nuclear pores [1]. An earlier survey of ribosomal proteins [2] indicated that most of eukaryotic ribosomal proteins carry either a basic cluster sequence or a basic bipartite sequence [3]. Conversely, there are exceptions as reported in yeast ribosomal proteins S22 and S25 [4], which use an alternated sequence to gain the nuclear entry. This suggests that ribosomal proteins may have a different NLS type. Additionally, most ribosomal proteins carry more than one NLS-like sequence and their real activity in respect to nuclear targeting have seldom been investigated. Human large ribosomal protein L7 represents a striking example, its sequence carries four basic cluster NLS-like type segments within the NH_2-region, and the number of these basic clusters in NH_2-region varies in homologous mammalian L7 proteins: there are six in the mouse and five in the rat [2,5]. So far, the function of these clusters in respect to nuclear targeting has never been experimentally determined. In addition, besides having a high RNA-binding nature [6], the repeated basic cluster sequence have been assumed to be the primary target of auto-antibodies [7], and to be frequently associated with nuclear-related auto-antibodies in auto-immune disease [8,9]. In this study, we have attempted to dissect the essential structure of human large ribosomal protein L7 in terms of nuclear targeting, the role in the functions of ribosome and that of ribosome assembly.

2. Materials and methods

2.1. Cloning and expressions of truncated L7 proteins

Plasmids that contain genes coded for full-length of human L7 protein were obtained from previous studies in this laboratory. The construction of the NH_2-truncated mutant L7 genes was carried out by the same PCR strategy using pGEM-T/L7 as the vector for a cytomegalovirus (CMV) promoter-driven plasmid.

2.2. Cellular localization of transiently expressed truncated L7 proteins

Plasmids that carried flag-tag L7 or mutant genes were transfected into HeLa cells that were seeded on a cover slide one day prior to the transfection. Cells were viewed at different time of post-transfection under a confocal fluorescence microscopy. Cellular localization of expressed flag-tagged L7 was determined using anti-flag antibody (M2, Eastman Kodak Co.) as the primary antibody. Detection of fluorescent was done by applying a second antibody of rhodamine-conjugated anti-mouse IgG antiserum, then exciting with a laser beam (568 nm wavelength).

2.3. Polysome assay

The polysome assay was carried out to detect the *in vivo* engagement of flag-tagged recombinant ribosomes in the protein synthesis. Briefly, embryonic kidney 293T cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum (Life Technologies, Inc.), 5 mM L-glutamine, 50 μg/ml streptomycin, and 50 U/ml penicillin. The cells were then transfected with CMV-driven plasmids that carried different flag-tagged L7 or truncated genes by a calcium phosphate procedure, and then the culture was continued in fresh medium. After 24 h post-transfection, cells (1×10^7 cells) were lysated and analyzed on a 12-ml 10–35% sucrose gradient containing buffer of 20 mM Tris–HCl, pH 7.6; 50 mM KCl and 3 mM $MgCl_2$. Gradients were centrifuged at 35,000 rpm for 165 min in Beckman SW41 rotor at 4 °C. Fractions (0.3 ml) were collected and monitored

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for absorbance at 254 nm wavelength to detect the standard polysome profile. Regions of subunits, monomers and polysome were subjected to the rRNA extraction and analyzed by 2% agarose gel electrophoresis.

For detection of recombinant protein in polyribosome, a modification of ribosomal proteins dot blotting was carried out. Polysome fractions containing ribosomal subunits, monomers and polysome, underwent total protein extraction with 67% acetic acid. The proteins were TCA-co-precipitated with 10 μg BSA, then spotted on nitrocellulose paper and analyzed for immuno cross-reactivity against anti-flag antibody.

3. Results

3.1. The structure within L7 participating in nuclear targeting

The long stretch of basic amino acid cluster at the NH_2-region of human ribosomal protein L7 has previously been suggested as potential basic cluster types of NLS [2,5,6]. To validate such a suggestion, genes coding mutant L7 proteins that have NH_2-terminal end deletion and other deletions were constructed with a flag tagged peptide adjacent to it (Fig. 1A).

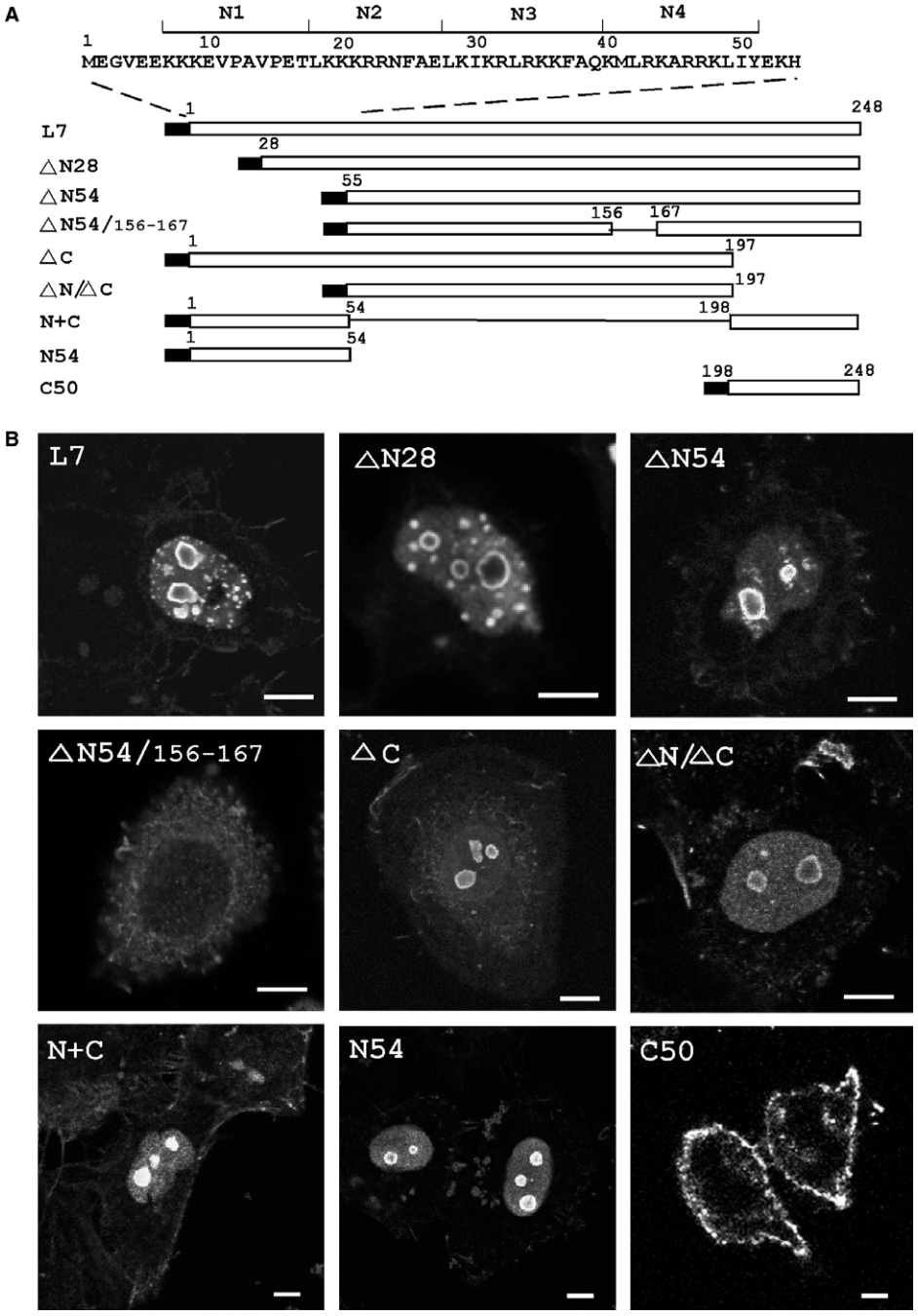

Fig. 1. Confocal analysis of sub-cellular localization of L7 protein and its mutant proteins. (A) The diagrams of mutant construction for this study. The first NH_2-terminal 54 amino acid residues are given and the possible basic cluster is showing in bold letters, N1, N2, N3, and N4 as suggested by Wool et al. (1995). The filled bar represents the flag-tagged peptide and the open bars are the remaining portions (as indicated by number) of L7. (B) The cellular distribution of flag-tagged L7 and mutant proteins at 24 h post-transfection viewed by immuo-fluorescent staining with a second FITC-conjugated antibody under a confocal microscopy. Anti flag-peptide antibody was used as the primary antibody. Bar = 5 μm.

The cellular localization of these transiently expressed flag-tagged L7 mutant proteins was observed at 24 h post-transfection using indirect immuno-fluorescent staining with anti-flag antibody under confocal microscopy. The results showed that protein having the NH_2-region or having part of the basic cluster sequence are all capable of reaching the nucleus and thence the nucleolus (Fig. 1B). This includes the peptide containing the first NH_2-terminal 54 amino acid residues (N54) and the chimeric protein (N + C) made up of a N54 peptide conjugated with a C50 peptide (Fig. 1B).

Under the same experimental procedure, truncated proteins without the NH_2-region (ΔN54), including mutant proteins missing both the NH_2-region and the last 50 amino acid residues of the C-terminal end (ΔN/ΔC), are also detected in nucleus and nucleolus (Fig. 1B), indicating that the middle segment could contain another NLS. Using a NLS computer search program (PredictNLS), the prospective NLS was suggested to be a bipartite NLS sequence at a region between residues 156 and 167. The authenticity of this region as a NLS was then confirmed by the deletion analysis. This is evident from the observation that a further deletion of that region in a recombinant mutant ΔN/ΔC excluded the protein from the nucleus in transfected cells (Fig. 1B).

3.2. Observation of L7-associated nuclear microbodies

Interestingly, when cellular localization was examined, many nuclear microbodies were found in association with the expressed recombinant L7 (Fig. 1B). These nuclear microbodies were also observed in association with the expressed mutant proteins ΔN28 and ΔN54, but not with any mutant proteins in which the C-region last 50 amino acid residues were missing (Fig. 1B). Apparently, having the C-peptide seems to be linked to the observed nuclear microbodies. Another intriguing finding is that the C-peptide actually localizes at the cellular membrane (Fig. 1B) when the C-peptide alone is transiently expressed in cells.

To further understand the nature of L7-associated nuclear microbody, a time-course evaluation of cellular distribution of fluorescent L7 in post-transfected cells was carried out. Four cellular distribution patterns of fluorescent L7 are generally observable in L7-transfected cells. They are: (a) cytosol only; (b) condensed at the nuclear membrane; (c) combined distribution at the nuclearplasm, nuclear microbodies, and nucleolus; and (d) concentrated at nucleolus (Fig. 2A). The percentage of each pattern cells was determined at different time intervals post-transfection and a possible trend representing how L7 routes to the nucleolus was derived. Apparently, the expression of flag-tagging L7 was detectable in cytoplasm as early as 4 h post-transfection. At 6 h, 12 h, and 24 h of post-transfection, most cell patterns, except the cytosolic one, were found (Fig. 2B). However, the percentage of transfected cells that have L7-associated nuclear microbodies displayed was different. This started to increase at 6 h of post-transfection, and reached to a peak at 12 h, then had decreased by 24 h post-transfection (Fig. 2B). At 24 h post-transfection, most of transfected cells belonged to nucleolar type (Fig. 2B). When the observation was further extended to 30 h post-transfection, the intensity of fluorescence in transfec-

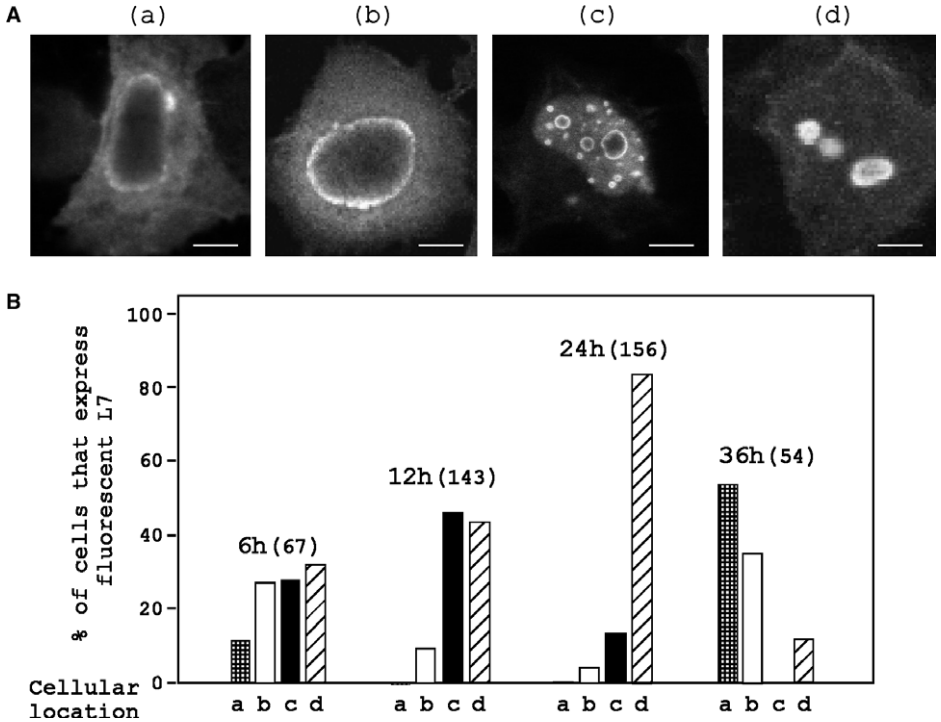

Fig. 2. Appearance of flag-tagging L7 in post-transfected cells at different time-intervals. (A) Fluorescent pattern of transfected cells with transiently expressed flag-tagging L7. Four types of fluorescent pattern were classified as depicted from overall observations among transfected cells at different time of post-transfection. HeLa cells were transfected with plasmid carrying flag-tagging L7 gene and examined as described. According to the cellular location and intensity of fluorescence, there are: (a) cytosol; (b) nuclear membrane; (c) nuclear foci and ring structure of nucleoli; and (d) condensed at nucleoli. (B) Distribution of L7 in cells observed at various time-intervals post-transfection. Only cells that carried flag-tagging L7 were scored. Number in the bracket representing the number of transfected cells survey at different time intervals.

ted cells became faint and most of detectable fluorescence was found to be at the rim of nuclear membrane or cytoplasm. Since the NH_2-terminal (where the flag peptide is tagged) is known to be exposed on surface of ribosome [10], the faint cytoplasm fluorescence might represent the staining of ribosomes at this late stage.

3.3. The structure within L7 participating in ribosome assembly

To map the essential structure of L7 involved in making the architecture of the ribosome, we transfected genes coding with a flag-tagged truncated protein, and monitored whether or not the expressed recombinant protein is present in ribosomes prepared from the corresponding transfected cells. It is understandable that ribosomes prepared from transfected cells would contain a mixed population of ribosomes composed of native ribosomes and recombinant ribosomes bearing a recombinant flag-tagged truncated protein if the recombinant proteins are able to be incorporated into ribosome through ribosome biogenesis in cells. In this respect, total ribosomal proteins were extracts from prepared ribosomes and examined for immuno-reactivity to anti flag antibody. The results from both immuno-dot blotting (Fig. 3A) and Western blotting (Fig. 3B) show that there was positive detection for all mutant proteins containing the middle section, including the mutant protein ΔN/ΔC, in their corresponding ribosome preparations. This suggests that neither the NH_2-region nor the C-region of L7, but only the middle section (minus the first 54 and the last 50 amino acid residues) are required for the structure of ribosome.

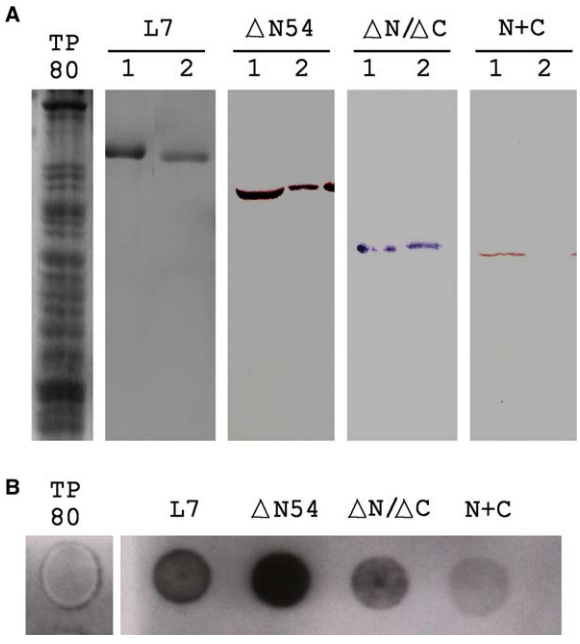

Fig. 3. Detection of recombinant mutant proteins in ribosomes. (A) Western blotting analysis of cell lysate (lane 1), and total protein extract of ribosome (lane 2) prepared from corresponding transfected cells. These proteins were separated by SDS-containing polyacrylamide gel electrophoresis, and immunologically detected using anti-flag antibody. (B) Dot blotting. The same total ribosomal protein extract that was immobilized on nitro-cellular paper and detected with anti-flag antibody. All samples are prepared from 24 h post-transfected cells with the corresponding plasmid carrying truncated L7 genes.

3.4. The structure within L7 participating ribosome function

Since the mutant proteins, ΔN54, ΔC50, and ΔN/ΔC, are capable of incorporating into the ribosome, the issue of whether these recombinant ribosomes are functionally active is of concern. To solve this issue, polyribosome analysis was carried out directly on the lysate of the corresponding transfected cells (Fig. 4A). After the regions 40S, 60S, 80S, and polysome were verified by rRNA analysis (Fig. 4B), the survival of the recombinant ribosome in polysome was examined by an immuno-dot blotting using an anti-flag antibody. The results (Fig. 4C) showed that only the recombinant ribosome bearing ΔN54 mutant protein could be detected as present in the polysome fraction (Fig. 4C), implying that ΔN54-recombinant ribosome was actively engaging in translation. The incorporation of a ribosome into a polysome, although it is normally considered to be a measure of ribo-

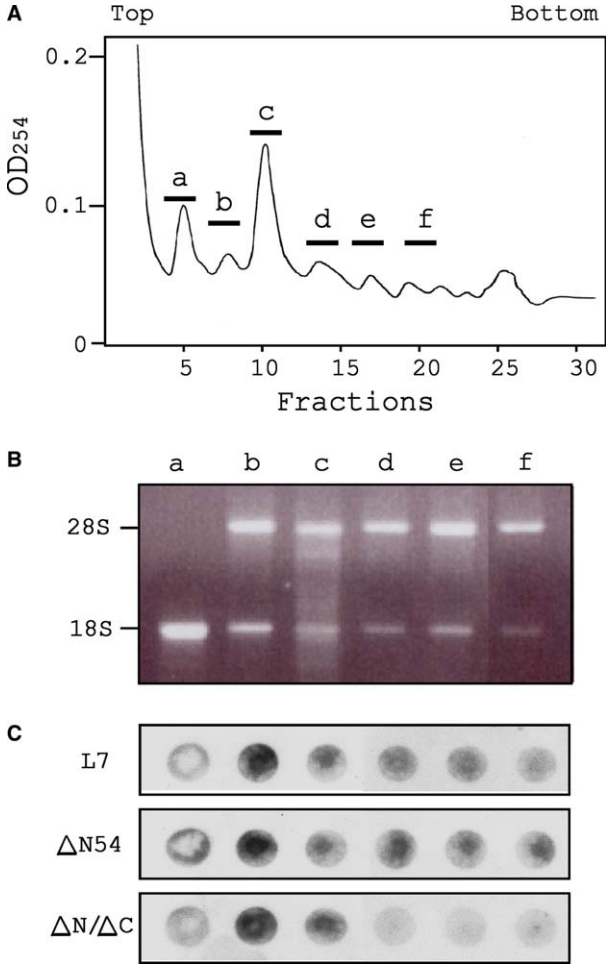

Fig. 4. Polysome analysis of recombinant ribosomes. (A) The representative polysome profile. The profile derived from cell lysate of 293T cells transfected with plasmid pCMV-flag-ΔN54 gene. No observable difference in polysome profile was found when the lysate from different transfected cells was analyzed. (B) Detection of rRNA in each pooled fraction. Total RNAs were extracted from the pooled fractions (indicated by a bar in panel A), and analyzed by 2% agarose gel. The corresponding ribosome subunits (40S, a; 60S, b), monomer (80S, c), and different sizes of polysome (d, e, and f) are indicated. (C) Detection of recombinant ribosomal protein that extracted from corresponding regions (a–f) by immuno-dot blotting assay with anti-flag antibody.

some functionality, in strict sense this does not prove this ribosome is driving the protein synthesis. Using polysome formation to depict the engagement of eukaryotic mRNA or translation factors in protein synthesis has been frequently reported [11,12] and is the normal assay. Accordingly, our data reconcile the dispensable nature of the NH_2-region, and the essentiality of C-region with respect to making a functional eukaryotic ribosome. Obviously, further research will be required to establish if the modified ribosome fully is capable of protein synthesis.

3.5. The NH_2-region of L7 is eukaryotic-specific and belongs to eukaryotic expansion segment

To try and understand essential nature of the middle structure, the C-region of L7, and the dispensable nature of NH_2-region of L7 to the eukaryotic ribosome, the genetic variability

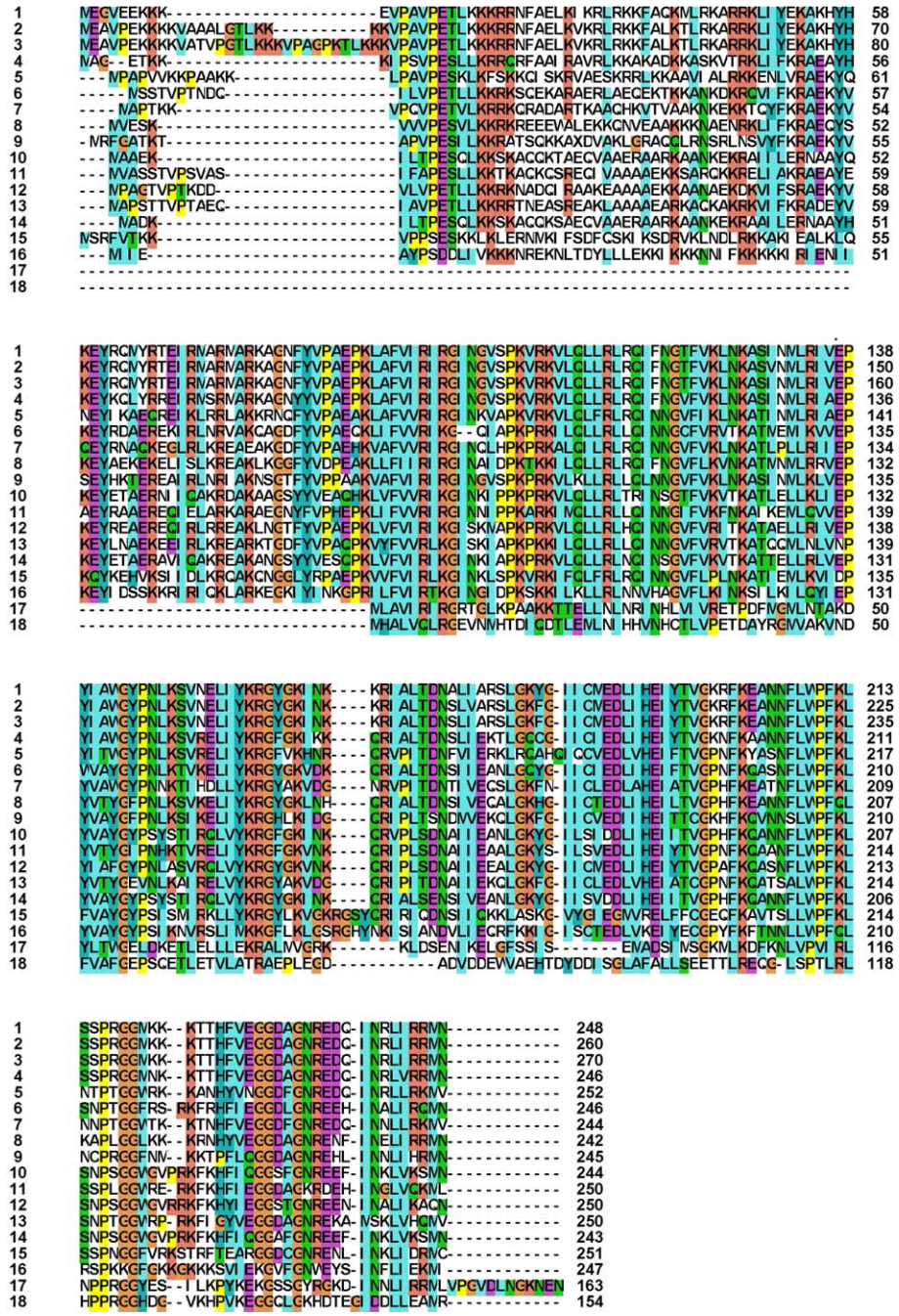

Fig. 5. The alignment of the primary sequence among homologous L7 proteins. (A) Sequence comparison of the primary structure of ribosomal protein L7 from (1) *Homo sapiens* (P18124); (2) *Rattus norvegicus* (P05426); (3) *Mus musculus* (P14148); (4) *Danio reri* (NP998809); (5) *Drosophila melanogaster* (P32100); (6) *Magnaporthe grisea* (XP359540); (7) *Caenorhabditis elegans* (001802C); (8) *Arabidopsis thaliana* (P60040); (9) *Dictyostelium discoideum* (P11874); (10) *Saccharomyces cervisiae* (P05737); (11) *Schizosaccharomyces pombe* (S25067); (12) *Yarrowia lipolytica* (Q6C603); (13) *Cryptococcus neoformans* (AAW41162); (14) *Eremothecium gossypii* (Q755A7); (15) *Cryptosporidium p* (EAK89575); and (16) *Guillardia theta* (C90133). (17) *Picrophilus torridus* (YP023439); and (18) *Haloacrcula marismortui* (X58395). The GeneBank accession number for each sequence is in parentheses. Each polar or non-polar residue is in a different color code for the comparison.

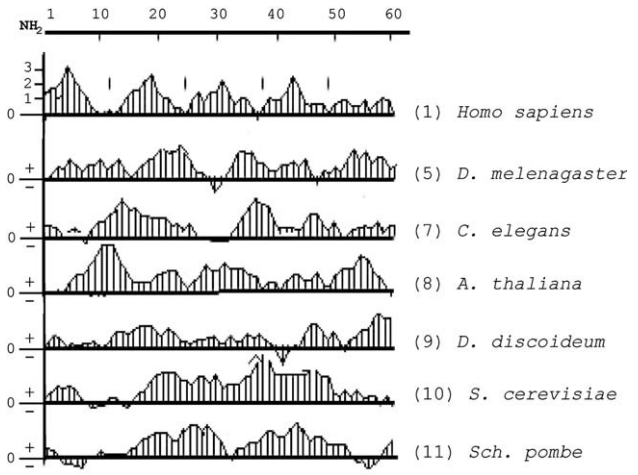

Fig. 6. The hydrophilic nature of the NH_2-region among homologous L7 proteins. The hydrophilicity of the first 60 amino acid residues of the sequence of L7 proteins from various organisms (Fig. 5) are predicted using the MACDNASIS Pro v1.0 program. Only seven representative sequences are shown. The positions of amino acid residues are given according to the sequence of the human L7. The degree of hydrophilicity indicates by a (+) values on the scale above the line, whereas, the hydrophobicity shows below the zero line with a (−).

in the primary and secondary structure among the L7 homologous proteins from different species was analyzed. The result (Fig. 5) shows that the C-region is highly conserved through out the archeal homologous protein, the divergence of which is similar to that obtained when the whole protein excluding the NH_2-terminal extension sequence is compared. In contrast, the sequence of the NH_2-terminal region among homologous of L7 is found to be vary, but its presences is essential in eukaryotes (Fig. 5). In fact, a common fundamental of having the NH_2-region is displaying when the secondary structure of the NH_2-region among eukaryotic species is analyzed (Fig. 6). The region is characteristically orientated into several distinct hydrophilic domains and these commonly existed in all the homologous L7 proteins in eukaryotes (Fig. 6). More strikingly, the essentiality of having the NH_2-region in eukaryotes is shown when one looks upon the primary sequence of the homologous protein from *Archeae* where the NH_2-terminal region is completely missing (Fig. 5, lane 18).

4. Discussion

In the view of archeal large subunit ribosome structure, certain ribosomal proteins have their domains buried deep within the structure, in a configuration that could only occur during folding of the rRNA [13–15]. Based on this, the archeal ribosomal protein L30, the homologue of L7 protein, has most of its residues involved in forming protein-binding pockets that bind bulged bases rRNA, suggesting that archeal L30 is an ''early'' protein in the assembly process [15]. Interestingly, the entire sequence of archeal L30 is found to aligned well with the sequence of middle segment plus the C-region of human L7 (Fig. 5). This finding provides good support for our claim that having the middle structure segment and the C-region of L7 are essential for making a functional ribosome, and the NH_2-region of L7 as an expansion segment that is unique to eukaryotes. The fact that the NH_2-region is exposed on surface of ribosome [10], and such a surface nature is in accord with the general description for all eukaryotic expansion segments [13,16,17], provides further support. Nevertheless, if the common existence of a structural domain among the homologous L7 proteins is considered, the question as to whether the NH_2-region in the L7 protein is important needs to be posed. Perhaps, the structural make-up of the NH_2-region sequence ensures targeting of L7 to nucleus as we demonstrated in this study, the structure may be important to RNA affinity [6] and protect the corresponding rRNA expansion segment or there may be an as yet unknown eukaryotic ribosome-specific purpose. These suggested propositions are currently under investigation.

The identification of the L7-associated microbody is of interest because the involvement of ribosomal protein in the pre-assembly process has seldom been reported. Recent proteomic data has also indicated [18] that not all ribosomal proteins are included in those pre-ribosomal particles. Our time-course evaluation of cellular distribution of L7 suggested that the L7-associated nuclear microbody was formed before the L7 protein entered nucleolus. The status of the observed L7-nuclear microbody does not seem to belong to 90S pre-RNP, nor does it fit with 60S pre-ribosomal particles, because it appears at nucleoplasm, whereas the 90S pre-RNP is located at the nucleolus [19], and the pre-60S is part of post-nucleolar ribosome [20,21]. Thus, such appearance of a L7-associated nuclear microbody at early stage of pre-nucleolar ribosome assembly might provide a clue as to how ribosomal proteins take part in ribosome assembly. Interestingly, in this study we have shown that the C-peptide of L7 carries membrane binding property, and is likely linked to the observed nuclear microbodies. Because the maturation of 40S has been reported to involve membrane-associated small ribosomal proteins [22,23], we speculated that the maturation of large ribosomal subunit might also involve a membrane binding property of L7, and the observed L7-associated nuclear microbodies might represent a complex that associates for ribosome assembly. The speculation is supported significantly by the fact that a ribosomal protein L7-related nucleolar protein in yeast is required for an early step in large ribosomal subunit biogenesis [24].

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References

[1] Talcott, B. and Moore, M.S. (1999) Getting across the nuclear pore complex. Trends Cell Biol. 9, 312–318.
[2] Wool, I.G., Chan, Y.L. and Gluck, A. (1995) Structure and evolution of mammalian ribosomal proteins. Biochem. Cell Biol. 73, 933–947.
[3] Dingwall, C. and Laskey, R.A. (1991) Nuclear targeting sequences – a consensus? Trends Biochem. Sci. 16, 478–481.
[4] Timmers, A.C., Stuger, R., Schaap, P.J., van't Riet, J. and Raue, H.A. (1999) Nuclear and nucleolar localization of *Saccharomyces cerevisiae* ribosomal proteins S22 and S25. FEBS Lett. 452, 335–340.
[5] Lin, A., Chan, Y.L., McNally, J., Peleg, D., Meyuhas, O. and Wool, I.G. (1987) The primary structure of rat ribosomal protein L7. The presence near the amino terminus of L7 of five tandem repeats of a sequence of 12 amino acids. J. Biol. Chem. 262, 12665–12671.

[6] von Mikecz, A.H., Ney, E., Krawinkel, U. and Hemmerich, P. (1999) Human ribosomal protein L7 carries two nucleic acid-binding domains with distinct specificities. Biochem. Biophys. Res. Commun. 258, 530–536.
[7] von Mikecz, A.H., Hemmerich, P.H., Peter, H.H. and Krawinkel, U. (1995) Autoantigenic epitopes on eukaryotic L7. Clin. Exp. Immunol. 100, 205–213.
[8] von Mikecz, A., Hemmerich, P., Peter, H.H. and Krawinkel, U. (1994) Characterization of eukaryotic protein L7 as a novel autoantigen which frequently elicits an immune response in patients suffering from systemic autoimmune disease. Immunobiology 192, 137–154.
[9] Neu, E., Hemmerich, P.H., Peter, H.H., Krawinkel, U. and von Mikecz, A.H. (1997) Characteristic epitope recognition pattern of autoantibodies against eukaryotic ribosomal protein L7 in systemic autoimmune diseases. Arthritis Rheum. 40, 661–671.
[10] Lin, A. (1991) Localization of surface peptide from ribosomal protein L7 on 80S ribosome by biotinylation. FEBS Lett. 287, 121–124.
[11] Brengues, M., Teixeira, D. and Parker, R. (2005) Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies. Science 310, 486–489.
[12] Chen, Z.Q., Dong, J., Ishimura, A., Daar, I., Hinnebusch, A.G. and Dean, M. (2006) The essential vertebrate ABCE1 protein interacts with eukaryotic initiation factors. J. Biol. Chem. 281, 7452–7457.
[13] Ban, N., Nissen, P., Hansen, J., Moore, P.B. and Steitz, T.A. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. Science 289, 905–920.
[14] Ramakrishnan, V. and Moore, P.B. (2001) Atomic structures at last: the ribosome in 2000. Curr. Opin. Struct. Biol. 11, 144–154.
[15] Klein, D.J., Moore, P.B. and Steitz, T.A. (2004) The roles of ribosomal proteins in the structure assembly, and evolution of the large ribosomal subunit. J. Mol. Biol. 340, 141–177.
[16] Dube, P., Bacher, G., Stark, H., Mueller, F., Zemlin, F., van Heel, M. and Brimacombe, R. (1998) Correlation of the expansion segments in mammalian rRNA with the fine structure of the 80S ribosome; a cryoelectron microscopic reconstruction of the rabbit reticulocyte ribosome at 21 A resolution. J. Mol. Biol. 279, 403–421.
[17] Morgan, D.G., Menetret, J.F., Radermacher, M., Neuhof, A., Akey, I.V., Rapoport, T.A. and Akey, C.W. (2000) A comparison of the yeast and rabbit 80S ribosome reveals the topology of the nascent chain exit tunnel, inter-subunit bridges and mammalian rRNA expansion segments. J. Mol. Biol. 301, 301–321.
[18] Takahashi, N., Yanagida, M., Fujiyama, S., Hayano, T. and Isobe, T. (2003) Proteomic snapshot analyses of preribosomal ribonucleoprotein complexes formed at various stages of ribosome biogenesis in yeast and mammalian cells. Mass Spectrom. Rev. 22, 287–317.
[19] Grandi, P., Rybin, V., Bassler, J., Petfalski, E., Strauss, D., Marzioch, M., Schafer, T., Kuster, B., Tschochner, H., Tollervey, D., Gavin, A.C. and Hurt, E. (2002) 90S pre-ribosomes include the 35S pre-rRNA, the U3 snoRNP, and 40S subunit processing factors but predominantly lack 60S synthesis factors. Mol. Cell. 10, 105–115.
[20] Nissan, T.A., Bassler, J., Petfalski, E., Tollervey, D. and Hurt, E. (2002) 60S pre-ribosome formation viewed from assembly in the nucleolus until export to the cytoplasm. EMBO J. 21, 5539–5547.
[21] Milkereit, P., Kuhn, H., Gas, N. and Tschochner, H. (2003) The pre-ribosomal network. Nucleic Acids Res. 31, 799–804.
[22] Sato, M., Saeki, Y., Tanaka, K. and Kaneda, Y. (1999) Ribosome-associated protein LBP/p40 binds to S21 protein of 40S ribosome: analysis using a yeast two-hybrid system. Biochem. Biophys. Res. Commun. 256, 385–390.
[23] Ford, C.L., Randal-Whitis, L. and Ellis, S.R. (1999) Yeast proteins related to the p40/laminin receptor precursor are required for 20S ribosomal RNA processing and the maturation of 40S ribosomal subunits. Cancer Res. 59, 704–710.
[24] Dunbar, D.A., Dragon, F., Lee, S.J. and Baserga, S.J. (2000) A nucleolar protein related to ribosomal protein L7 is required for an early step in large ribosomal subunit biogenesis. Proc. Natl. Acad. Sci. USA 97, 13027–13032.